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SHORT COMMUNICATION

Association of prolactin and beta-lactoglobulin genes with milk production traits and somatic cell count among Indian Frieswal (HF × Sahiwal) cows

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Abstract Prolactin (*PRL*) and beta-lactoglobulin (*BLG*) are two important candidate genes well known to be associated with milk production traits as well as somatic cell count (SCC) among dairy cattle breeds. In the present study, the intron 3 of *PRL* and the spanning region between exon IV and intron IV of the *BLG* gene were chosen for genotyping and their association with milk production traits as well as SCC among HF crossbred cattle (i.e., Frieswal) that originated from India. We observed that the AA genotype frequency of *PRL* among Frieswal cows is higher than that of AB and BB. Our findings showed that cows with AA and BB genotypes had significantly ($p < 0.05$) higher total milk yield and peak yield than AB genotype cows. Comparing SCC with various genotypic groups, we observed that BB genotype cows had significantly ($p < 0.05$) lower SCC than those with AB and AA genotypes. In the case of *BLG*, the genotypic frequency of BB was higher than that of AB and AA. The AB and BB genotypes of *BLG* had a significant ($p < 0.05$) effect on total milk yield and peak yield compared with AA. The SCC of the AA genotype of BLG is significantly ($p < 0.05$) lower than that of AB and BB. This study thus indicates that AA and BB genotypes in *PRL* as well as AB and BB genotypes in *BLG* may be more suitable for better milk production; however, cows having BB genotype in *PRL* and AA genotype in *BLG* may show more resistance to mastitis than those with other genotypes among HF crossbred cattle.

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Introduction

Prolactin (*PRL*) is known to have diverse biological functions such as water and electrolyte balance, growth and development, as well as immune and reproductive function.[1] Bovine *PRL* is located on chromosome 23, which is composed of five exons and four introns.[2] Many studies in relation to *PRL* gene polymorphism were conducted earlier[2,3] and reported that polymorphic loci may be associated with milk production and quality traits.[4,5] Similarly, beta-lactoglobulin (*BLG*) is a milk whey protein that constitutes about 14% and 53% of the total milk protein and total whey protein, respectively.[6] The *BLG* locus was reported to have a significant influence on cheese yield, indicating the higher yield by heterozygotes.[7]

Somatic cells are mastitis indicators that can be used for monitoring the level of subclinical mastitis. With the advancement of molecular marker-based selection, screening of novel genes and their association studies with somatic cell count (SCC) need to be accomplished. There are several reports suggesting that various candidate genes, viz., *TLR2*,[8] *BoLA*,[9] *IL8*,[10] *CXCR2*,[11] *TLR4*,[12] and *CACNA2D1*[13], are associated with the occurrence of mastitis among different cattle breeds. Similarly, earlier findings also revealed that bovine *PRL* and *BLG* are associated with SCC among dairy cattle breeds.[14–17] In the present context, our aim was to associate the *PRL* and *BLG* genes with milk production traits as well as SCC among Frieswal (HF × Sahiwal) crossbred cattle developed in India.

Materials and methods

Sample collection and genomic DNA extraction

The cattle resource population of the present study involved 126 randomly selected Frieswal (HF × Sahiwal) crossbred cows maintained at the Military Farm, Meerut, Uttar Pradesh, India. Blood samples were collected and genomic DNA was extracted using standard phenol chloroform method.[18]

Polymerase chain reaction amplification and genotyping

The *PRL*—*Rsa*I genotypes were analyzed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. A 156-bp fragment of intron 3 of *PRL* gene was amplified with PCR using forward (5′-CGAGTCCTTATGAGCTTGATTCTT-3′) and reverse (5′- GCCTTCCAGAAGTCGTTTGTTTTC-3′) primers.[2] A 398-bp fragment of the *BLG* gene spanning over part of exon IV and intron IV (104 bases of exon IV and 294 bases of intron IV) was amplified using a pair of forward (5′-CGAGAACAAAGTCCTTGTGCT-3′) and reverse (5′-CCGGTAACAAAGGCTGTTAGA-3′) primers.

A 25-μL reaction volume containing 50—100 ng DNA template, 10 pM of each primer, 100μM each dNTP, 1.5mM $MgCl_2$, 1 U Taq DNA polymerase, and 10× PCR assay buffer (Bangalore Genei Pvt. Ltd., Bangalore, India) was set up for each individual animal. The cycling condition for *PRL* gene amplification were as follows: denaturation −94°C for 5 minutes, followed by 30 cycles: denaturation −94°C for 30 seconds, primer annealing −55°C for 30 seconds, PCR products synthesis −72°C for 1 minute, and final synthesis −72°C for 8 minutes. Amplified DNA was digested by *Rsa*I (*PRL*) and *Hae*III (*BLG*) enzyme at 37°C for 4 hours with the following reaction mixture: PCR product (8 μL), buffer (2.5 μL), enzyme (0.5 μL), and dH_2O (4 μL). The digestion products were separated by electrophoresis in 2.5% agarose gels in 1× Tris-borate-EDTA.

Screening of animals for mastitis and SCC

Cows were first screened using the California Mastitis Test (described elsewhere). The SCC was done as per the standard protocol described earlier.[19] Milk samples were thoroughly mixed, and 10 μL of milk was taken on a grease-free glass microslide. The milk was uniformly smeared and air-dried. The smear was stained with modified Newman—Lampert stain and was examined using oil immersion lens (100×). Somatic cell was counted in selected 30 fields from each slide under the microscope. The average number of cells in all 30 fields were multiplied by the working factor of the microscope and expressed as SCC per milliliter of milk. SCC was converted into somatic cell score (SCS), using the following formula:

$$SCS = \log 2(SCC/100{,}000) + 3$$

Statistical analysis

Data are presented as LS mean ± SE and analyzed using the SPSS version 10.0 statistical program (SPSS, Inc., Chicago, IL, USA). Significant differences of the genotype effect on milk production traits and SCC were determined with analysis of variance using the SPSS program. The following model was used to test the effect of genotypes on milk production traits:

$$Y_{ij} = \mu + G_i + e_{ij}$$

where Y_{ij} is the effect of the jth individual belonging to the ith genotype, μ is the overall mean for traits, G_i is the effect of the ith genotype, and e_{ij} is the residual error.

The analysis of associations between the genotypes and SCS that reflects mastitis traits was carried out using the following model:

$$Y_{ijK} = \mu + SE_i + G_j + P_k + e_{ijk}$$

where Y_{ijK} is the observation of first cow's SCC within having kth genotypes, μ is the global mean, SE_i is the fixed effect of season calving, G_j is the fixed effect of genotypes, P_k is the fixed effect of parity, and e_{ijk} is the residual error.

Results and discussion

Our results demonstrated that exon 3 *PRL*—Rsa*I* loci are polymorphisms among the studied Frieswal population (Fig. 1). The *PRL* genotype variants obtained in the present study revealed that the frequency of the AA genotype was higher among Frieswal cattle (Table 1). Our results support the earlier findings by several researchers in different regions of the world, with different breeds and sample

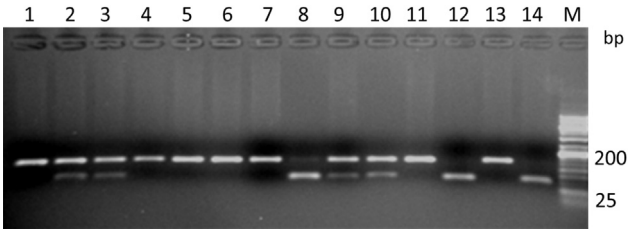

Figure 1 Polymerase chain reaction—restriction fragment length polymorphism (PCR—RFLP) gel electrophoresis of prolactin gene showing genotypes AB (lanes 2, 3, 8, 9, and 10), AA (lanes 1, 4, 5, 6, 7, 11, and 13) and BB (lanes 12 and 14).

sizes.[4,20,21] When analyzing the genotypes favorable for milk production traits, we observed that genotypes AA and BB had significantly ($p < 0.05$) higher total milk yield and peak yield compared to BB (Table 2). Dybus et al[2] found that genotype AA was favorable for the second and third lactations, whereas genotype AB was in the first lactation in Jersey cattle, and both genotypes AA and AB were favorable in Black and White cattle. Other authors[21] indicated that genotype AB in Black Pied cattle affected milk, fat, and protein production, whereas in the case of Red Pied cattle genotype BB was favorable. Chrenek et al[22] examined the influence of polymorphism of *PRL—RsaI* in Brown Swiss cattle and found no significant differences among cows with diverse *PRL* genotypes. Our finding thus may indicate that the AA genotype of the *PRL* gene is suitable for selecting HF cross for better milk traits. The association analysis of SCC revealed that the BB genotype of *PRL* has a significantly ($p < 0.01$) lower SCC compared to AB and AA genotypes, and the results support the earlier findings observed by Maksymiec et al.[14]

The different kinds of genotypes observed for *BLG* among Frieswal cattle are shown in Fig. 2. The genotypic frequency was highest for genotype BB followed by AB and AA (Table 1), which is in agreement with the other findings reported by Zhou et al[23] and Kim et al[24] among Holstein Friesian and Hanwoo cattle, respectively. The *BLG* locus was reported to have a significant influence on cheese

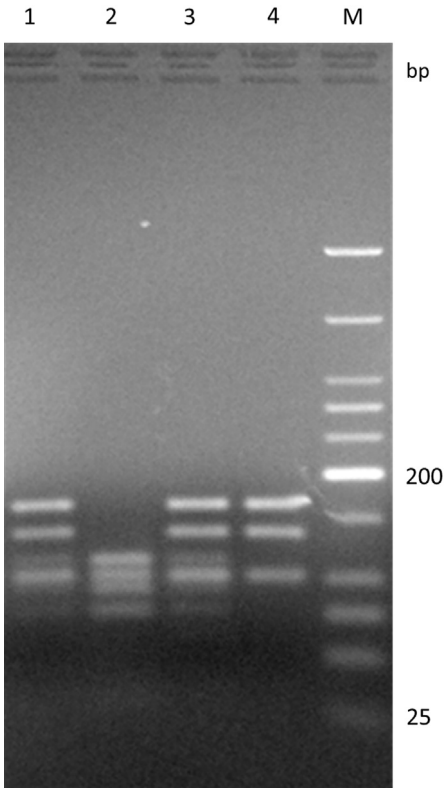

Figure 2 Polymerase chain reaction—restriction fragment length polymorphism (PCR—RFLP) gel electrophoresis of beta-lactoglobulin gene showing genotypes AA (lane 4), BB (lane 2), and AB (lanes 1 and 3).

Table 1 Calculated genotype and allele frequencies of prolactin (*PRL*) and beta-lactoglobulin (*BLG*).

Genes	Genotype frequency ($N = 126$)			Allele frequency	
	AA	AB	BB	A	B
PRL	0.595 ($n = 75$)	0.310 ($n = 39$)	0.095 ($n = 12$)	0.750	0.250
BLG	0.238 ($n = 30$)	0.286 ($n = 36$)	0.476 ($n = 60$)	0.381	0.619

Table 2 Associations between the prolactin (PRL) genotypes with milk yield traits and somatic cell count among Frieswal cow milk.

Traits	Genotypes (n)		
	AA (75)	AB (39)	BB (12)
Total milk yield*	2665.97 ± 161.91[a]	2211.40 ± 296.52[b]	2550.80 ± 166.4[a]
Peak yield*	13.80 ± 12.51[a]	11.93 ± 11.12[b]	13.29 ± 7.17[a]
SCC of milk*	3.79 ± 2.12[a]	3.42 ± 1.95[a]	2.013 ± 1.68[b]

Data presented in parentheses are the total number of animals. Least square (LS) means bearing different superscripts in a row indicate statistically significant differences.
* Significant at 5% level ($p < 0.05$).
SCC = somatic cell score.

Table 3 Associations between the beta-lactoglobulin (BLG) genotypes with milk yield traits and somatic cell count among Frieswal cow milk.

Traits	Genotypes (*n*)		
	AA (30)	AB (36)	BB (60)
Total milk yield*	2257.74 ± 151.45[a]	2678.36 ± 198.96[b]	2514.39 ± 136.72[b]
Peak yield*	11.43 ± 6.69[a]	13.81 ± 8.02[b]	12.91 ± 6.55[b]
SCC of milk*	2.12 ± 1.63[a]	3.98 ± 1.13[b]	3.31 ± 1.98[b]

Data presented in parentheses are the total number of animals. Least square (LS) means bearing different superscripts in a row indicate statistically significant differences.
* Significant at 5% level ($p < 0.05$).
SCC = somatic cell score.

yield, indicating the higher yield by heterozygotes.[7] Jairam and Nair[25] revealed that cows with AB genotype had a lower age at first calving. Weight at birth to 12 months of age was also found to be influenced by *BLG* loci.[26] However, Ng-Kwai-Hang et al[27] found no association of milk protein types with days to attain first breeding, days open, and number of service per conception. In the present study, we observed that the AB and BB genotypes of *BLG* had a significant ($p < 0.05$) effect on total milk yield and peak yield than AA genotypes (Table 3). Thus, the AB and BB genotypes may be favorable among Frieswal cows for better milk production traits. *BLG* polymorphism has previously been associated with SCC in dairy cows.[15,17,28] This gene has three genetic variants—A, B, and C—in sheep,[16] with alleles A and B being the most prevalent. Kriventsov et al[17] have reported a lower total milk microbiota in animals producing *BLG* B than in animals expressing *BLG* A. Walawski et al[28] found an association between genotype AA for the *BLG* gene and a high level of SCC in cows. However, Luhar et al[15] found that allele B is associated with mastitis. In the present study, we have observed that the SCC of cows with genotype AA is significantly ($p < 0.05$) lower than that of cows with genotypes AB and BB (Table 3), and thus the AA group of animals may be favorable for selecting mastitis-tolerant animals.

Conclusion

The information provided in the present study will be very useful for improving milk production traits and mastitis resistance in dairy cattle by marker-assisted selection.

Conflicts of interest

The authors do not have any conflicts of interest to declare with regard to the present study.

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